Examining the Temporal Course of Over-generalized Conditioned Threat Expectancies in Posttraumatic Stress Disorder Using a Nonparametric Regression Model

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Abstract

One key conditioning abnormality in posttraumatic stress disorder (PTSD) is heightened generalization of fear from a conditioned danger-cue to similarly appearing, safe stimuli. Such over-generalization is often assumed to be a stable feature of PTSD, yet several lab-based conditioning findings suggest that over-generalization in PTSD can be reduced with sufficient learning trials. The present study represents the first effort to track the trial by trial timecourse of heightened generalization in PTSD with the prediction of heightened PTSD-related over-generalization in earlier trials that reduces toward the end of the learning record. Combat veterans with PTSD (n = 15), subthreshold PTSD (SubPTSD: n = 18) and trauma controls (TC: n = 19) completed a conditioned fear-generalization task. Trial by trial group differences in generalized perceived risk of electric shock were assessed to three classes of (safe) generalization stimuli parametrically varying in similarity to a conditioned danger-cue paired with electric shock. Data were analyzed using nonparametric regression. Results demonstrated those with PTSD and SubPTSD, relative to TC, displayed elevated generalization to all generalization stimuli combined, in early but not late learning trials. Over-generalization in PTSD and SubPTSD also persisted across trials to a greater extent for classes of generalization stimuli bearing higher resemblance to the conditioned danger-cue. Current findings support the use of prolonged courses of exposure therapy in PTSD that maximize violations of threat-related expectancies for safe stimulus-events resembling the traumatic encounter, especially as safe stimulus-events increase in similarity to trauma-related threat cues.

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Introduction

Generalization of conditioned fear is the process by which fear of a conditioned stimulus (CS+) paired with an aversive, unconditioned stimulus (US) transfers to resembling, safe stimuli (Pavlov, 1927). Evidence linking conditioned fear generalization to maladaptive anxiety dates back to Watson and Rayner (1920), who demonstrated generalization of conditioned fear to all things furry in a toddler ("Little Albert") following acquisition of fear-conditioning to a white rat. Heightened generalization of conditioned fear has since been adopted as a core feature of posttraumatic stress disorder (PTSD; Ehlers & Clark, 2000; Foa, Steketee, & Rothbaum, 1989) through which fears of people, places, and things associated with trauma unduly extend to safe situations "resembling" the traumatic encounter (American Psychiatric Association, 2000, 2013). Over-generalization contributes to PTSD symptomatology by unnecessarily increasing the number of innocuous stimuli in the individual's post-trauma environment capable of eliciting and maintaining trauma-related distress.

In support of the link between PTSD and heightened levels of generalization, a meta-analysis of 13 lab-based discriminative conditioning studies in PTSD found elevated acquisition of fear to conditioned safety-cues (CS-) bearing perceptual resemblance to CS+ among those with versus without PTSD (Duits et al., 2015), a finding consistent with over-generalization of fear from CS+ to CS- in PTSD patients. Furthermore, PTSD-related over-generalization of conditioned fear has recently been documented by studies applying systematic generalization methods to elicit *generalization gradients*: declines in conditioned responding as presented stimuli

incrementally differentiate from CS+, with more shallow gradients indicative of over-generalization found among those with PTSD (Kaczkurkin et al., 2017; Lissek & Grillon, 2012; Morey et al., 2015). Similar effects of over-generalization have been found by generalization gradient studies in panic disorder (Lissek at al., 2010) and generalized anxiety disorder (Cha et al. 2014; Greenberg, Carlson, Cha, Hajcak, Mujica-Parodi, 2013; Lissek et al., 2014, but also see Tinoco-González et al., 2015), implicating generalized conditioned-fear as a transdiagnostic marker of anxiety- and trauma-related disorders.

To date, the expression of over-generalized conditioned fear has largely been viewed as a stable clinical feature of anxiety and trauma-related pathology, yet results from several studies suggest that such over-generalization may dissipate given sufficient learning trials. For example, two past discriminative fear-conditioning studies found a lack of discrimination between CS+ and a conditioned safety-cue (CS-) among those with PTSD (Grillon & Morgan, 1999) and panic disorder (Lissek et al., 2009) in early, but not later stages of conditioning, driven by enhanced fear to CS-. Similarly, the above mentioned meta-analysis of 13 conditioning studies in PTSD (Duits et al., 2015) found that enhanced fear, among those with PTSD relative to controls, to CS- bearing resemblance to CS+ was restricted to the acquisition training phase and did not emerge during the subsequent extinction phase. That is, PTSD patients displayed heightened fear reactivity to the first half (acquisition) but not the second half (extinction) of unreinforced CS- trials. Importantly, response to the CS+ was significantly higher for those with PTSD than the healthy controls during extinction (and was not during acquisition),

demonstrating that reduced fear to the CS- was not due just to overall reduction of fear response for individuals with PTSD. Further evidence of delayed learning has also been found in studies assessing trial by trial extinction of conditioned fear in traumatized samples. These studies have found elevated fear to (unreinforced) CS+ during early and middle, but not later extinction trials among those with PTSD (Fani et al., 2012; Norrholm et al., 2011; Norrholm et al., 2013, but see Orr et al., 2000) and trauma survivors with more severe re-experiencing symptoms (Norrholm et al., 2015). Taken together, these findings suggest that over-generalization to safe stimuli resembling danger cues among those with PTSD may reduce to healthier levels given a sufficient number of learning trials. However, no study to date has applied the generalization gradient methodology to assess the trial by trial course of over-generalization in PTSD.

In the present study, we aim to fill this gap by reanalyzing previously published data (Kaczkurkin et al., 2017) with a nonparametric regression model to examine the within-session temporal course of group differences in generalized conditioned threat expectancy across military veterans with PTSD, subthreshold PTSD (SubPTSD), and no PTSD (trauma controls: TC). Though Kaczkurkin and colleagues (2017) report effects of PTSD on generalization with both behavioral and fMRI measures, the current effort looks only at behavioral indices because behavioral, but not fMRI, data have sufficient reliability for analyses at the individual trial level. Behavioral data included trial by trial online ratings of shock-US expectancy to CS+ (10 trials), two CS- (10 trials each), and three generalization stimuli (GS: 10 trials each) that together form a continuum-of-

similarity across CS+, GSs, and CS-. Based on previous findings, we predicted that those with PTSD and SubPTSD, relative to TC, would show: 1) similarly high levels of generalization in early trials when all groups are learning the signal value of GSs; 2) over-generalization toward the middle of trials; and 3) similarly low levels of generalization by the end of the learning record.

Methods

Participants

The study sample consisted of participants from a previous study (Kaczkurkin et al., 2017) for which trial by trial conditioning data were available for analysis. Participants were 61 male, United States combat veterans from Operation Iraqi Freedom and Operation Enduring Freedom. All veterans were assessed using the Clinician Administered PTSD Scale for the DSM-IV (CAPS: Blake et al., 1995). If a participant did not meet criteria for PTSD, they were placed into either SubPTSD (CAPS score: 20-39) or trauma control (TC) groups (CAPS score: 0-19) based on previous recommendations (Weathers, Keane, & Davidson, 2001). Psychiatric co-morbidities were assessed with the Structured Clinical Interview for the DSM-IV (SCID-I; First, Gibbon, Spitzer, & Williams, 2001) . Nine participants were excluded from analyses because they failed to learn the CS+/US contingency (as indicated by an average CS+ versus CS- risk rating difference ≤ 0). Final analyses included 15 PTSD, 18 SubPTSD, and 19 TC participants. Full exclusion criteria and sample characteristics are described in Appendix A (text and Table AA1). The study was approved by the University of Minnesota and

Minneapolis VA Medical Center IRBs and all subjects completed informed consent before participating. All participants were compensated for their time.

Generalization Task

The generalization task consisted of five checkerboard textured rings that parametrically varied in size (i.e., conditioned danger-cue [CS+], three classes of generalization stimuli [GS_3, GS_2, GS_1], and conditioned safety-cue [oCS-]), as well as one "V" shaped conditioned safety-cue (i.e., vCS-; see Figure 1). The CS+ is the largest ring for counterbalance Group A and the smallest ring for counterbalance Group B. The vCS- served as a control condition to assess broader generalization to all things circular. The stimuli were of checkerboard texture and flickered at a rate of 10Hz for a separate, retinotopic mapping project; however, the only important characteristics of the stimuli for the present study are their size and shape.

The task included three phases: 1) *Pre-acquisition*: 20 of each stimulus type (CS+, GS_3, GS_2, GS_1, oCS-, vCS-) presented without shock, 2) *Acquisition*: 15 CS+, oCS-, and vCS-, where 12 of CS+ stimuli co-terminated with shock (100ms 3-5 mA, administered to the right ankle), and 3) *Generalization*: 20 of each stimulus type (CS+, GS_3, GS_2, GS_1, oCS-, vCS-) with an additional 10 shock-reinforced CS+ administered to prevent extinction of conditioned fear. Importantly, stimulus types were presented in the same order for every participant, rendering data conducive to trial by trial analyses. The stimuli were presented for 4 seconds each in quasi-random order so that no stimulus was presented more than twice in a row. For the Generalization phase, there were 10 blocks,

each containing 13 trials (2 of each stimulus type plus one additional reinforced CS+). Inter-trial intervals ranged between 2.4-4.8 seconds.

In terms of counterbalancing, there were 7 PTSD, 9 SubPTSD, and 8 TC participants in counterbalance A (largest circle as CS+) and 8 PTSD, 9 SubPTSD, and 11 TC participants in counterbalance B (smallest circle as CS+). For each phase, participants were instructed to monitor a stream of color-changing cross-hairs (5 colors: blue, yellow, red, green, purple) in the middle of the stimuli presented on screen, which was a task developed to maintain their gaze in the center of the screen (Schwartz et al., 2005). Colors changed every 800 ms. Participants were asked to quickly rate their perceived risk of shock (0 = *no risk*, 1 = *moderate risk*, and 2 = *high risk*) each time a crosshair in the middle of the presented stimulus turned red, using a three-button response pad (Lumina LP-404 by Cedrus). The red crosshair appeared on half of all trials for each phase; thus, it appeared 8 times for each stimulus-type at the Acquisition phase and 10 times for each stimulus-type during Pre-acquisition and Generalization phases. Additional task parameters are described in Kaczkurkin et al. (2017).

Procedure

Participants were not told the CS/US and GS/US contingencies; however, they were told that they may learn when shock would occur if they attended to the shapes presented on the screen during the task. Shock electrodes were attached before the participant entered the MRI. A shock workup procedure was completed, where 1-3 sample shocks were given and adjusted to obtain a level of shock that the participants rated as 'highly uncomfortable or mildly painful'. After the shock workup, participants

practiced responding to the red crosshairs appearing in the center of the stimuli using the button box. After the practice, participants were placed in the scanner, and structural scans were acquired followed by Pre-acquisition, Acquisition, and Generalization phases.

Data Analysis

Nonparametric regression. The trial by trial time course of group effects on acquisition and generalization of perceived risk were analyzed with a nonparametric mixed-effects regression model (Gu & Ma, 2005; Helwig, 2016; Wang, 1998a, 1998b; Zhang et al., 1998). The model was a three-way (Group x Stimulus-type x Trial) smoothing spline analysis of variance (SSANOVA: Gu, 2013; Wabha, 1990). While parametric regression assumes an exact mathematical model of the relationships between predictor variables and a response variable and aims to test hypotheses about model parameters (e.g., Faraway, 2014), nonparametric regression provides data driven models of relationships among variables of interest (e.g., Gu, 2013; Helwig & Ruprecht, 2017). Because the mathematical function that best describes PTSD-related differences in rates of learning across generalization trials is unknown, a nonparametric approach was selected. Smoothing splines in our model are selected via cross-validation (Craven & Wahba, 1978), which aim to reduce the overfitting that can be problematic with small sample sizes. Additional information on smoothing splines can be found in Appendix C.

SSANOVA Model. Predictor variables entered into the SSANOVA model included: 1) trial (8 levels: trials 1-8), group (3 levels: PTSD, Sub-PTSD, TC), and stimulus-type (3 levels: vCS-, oCS-, CS+) for the Acquisition phase; and 2) trial (10 levels: trials 1-10), group (3 levels: PTSD, Sub-PTSD, TC), and stimulus-type (6 levels:

vCS-, oCS-, GS_1, GS_2, GS_3, CS+) for the Generalization phase. We used the following model form,

$$y_{its} = f(t, g_i, s) + u_i + \epsilon_{its}$$

where y_{its} is the observed risk rating for the i-th subject for the t-th trial and s-th stimulus. $f(\cdot)$ denotes some unknown function to be estimated from the data, $u_i \sim$ N(0, $\sigma^2_{g_i}$) denotes a random intercept (i.e., baseline risk appraisal) that is unique to each subject (with group specific variance terms for the present analysis), and $\epsilon_{its} \sim$ N(0, σ^2) denotes the model error term, which is assumed to be independent, identically distributed, and independent of u_i terms. The function $f(\cdot)$ outputs the estimated risk from the input combination of trial (t), group (g), and stimulus (s). The model mean function above can be decomposed as

$$f(t, g_i, s) = f_0 + f_T(t) + f_G(g_i) + f_S(s) + f_{TG}(t, g_i) +$$
$$f_{TS}(t, s) + f_{GS}(g_i, s) + f_{TGS}(t, g_i, s)$$

where f_0 is the constant, intercept term, f_T is the main effect of trial, f_G is the main effect of group, f_S is the main effect of stimulus, f_{TG} is the trial by group interaction effect, f_{TS} is the trial by stimulus interaction effect, f_{GS} is the trial by stimulus interaction effect, and f_{TGS} is the three-way interaction effect among trial, group, and stimulus. Note that

$$f(t, g_i, s) = \hat{y}_{gts}$$

where $\hat{y}_{gts}$ is our model's estimated risk appraisal for a given group (g), at a given trial (t), for a given stimulus type (s).

The model was fit using the *bigssp* function in the "bigsplines" package (Helwig, 2018) in R (R Core Team, 2018), which estimates variance and smoothing parameters

using the two-stage approach described in Helwig (2016). The two-stage approach estimates the smoothing parameters via generalized cross-validation (Craven & Wahba, 1978) after estimating the variance parameters via restricted maximum likelihood estimation (Patterson & Thompson, 1971). The argument "skip.iter" was set to True, which skips the iterative smoothing parameter update; it was set to True since it is more computationally efficient and the fitted values showed relative stability when "skip.iter" was set to both True and False. The present model uses 72 knots for the Acquisition phase (there are 3 groups x 8 trials x 3 stimuli; a total of 72 unique combinations of predictor variable values) and 180 knots for Generalization (there are 3 groups x 10 trials x 6 stimuli; a total of 180 unique combinations of predictor variable values). Basic information on knots, knot selection, and knot placement can be found in Gu (2013) and James et al. (2013, pg. 274). Trial, group, and stimulus were modeled using cubic, nominal, and ordinal smoothing splines, respectively. More information on types of smoothing splines can be found in Gu (2013) and Helwig (2017).

Contrasts. The SSANOVA results were used to form two types of effect contrasts (Helwig, Shorter, Ma, and Hsiao-Wecksler, 2016): stimulus contrasts and group-stimulus contrasts.

Stimulus contrasts. A stimulus contrast takes the estimated risk appraisal for a given group, trial, and stimulus (e.g., PTSD group's estimated risk appraisal at trial 1 for vCS-) from the SSANOVA model and subtracts it from the estimated risk appraisal for the *same* group at the *same* trial, but for a *different* stimulus (e.g., PTSD group's estimated risk appraisal at trial 1 for GS_3). The stimulus contrasts (SC) take on the form

$$SC = f(Trial, Group, StimulusA) - f(Trial, Group, StimulusB).$$

The vCS- was used as a non-circular control stimulus to compare against all other generalization stimuli (i.e., the vCS- was Stimulus B in Equation 2 above for *all* Generalization phase contrasts); in other words, the vCS- was used to control for fear unrelated to the circular shape. Increased generalization was defined by increases in stimulus contrasts between the three circular GSs (GS_3, GS_2, and/or GS_1) relative to the non-circular vCS-. Increases in discrimination between CSs was defined by increases in stimulus contrasts between the CS+ relative to the oCS- and non-circular vCS-. The goal of the stimulus contrast analyses was to model the degree to which each separate group generalized the GSs across the course of the Generalization phase. We also examined how discrimination of the CS+ vs. vCS-, CS+ vs. oCS-, and oCS- versus vCS- changed throughout the course of the Acquisition phase.

More specifically, we computed stimulus-type contrasts using risk appraisal estimates from our fitted SSANOVA model to find contrast estimates of: 1) CS+ minus vCS− and CS+ minus oCS− and oCS− minus vCS− at each of 8 trials for each of the 3 groups during the Acquisition phase, and 2) all three GS estimates averaged minus vCS− *and* each separate stimulus type (CS+, GS3, GS2, GS1, oCS−) minus vCS− at each of 10 trials for each of the 3 groups during the Generalization phase. While all GSs averaged minus vCS− captured the time-course of overall generalization, each GS minus vCS− reflected levels of generalization for GSs with high (GS_3), moderate (GS_2), and low (GS_1) resemblance to CS+. We also found contrast estimates of CS+ minus oCS- and CS+ minus vCS- for the Generalization phase.

Group-stimulus contrasts. The group-stimulus contrasts are an extension of the stimulus contrasts. Group-stimulus contrasts tested *group differences* in trial by trial stimulus contrasts to compare levels of generalization and discriminative conditioning across each pair of groups. For these contrasts, over-generalization was defined as stronger generalization, captured by the size of stimulus contrasts, in the PTSD or SubPTSD group versus the TC group. The group-stimulus contrasts (GSC) take on the form

$$GSC = f(Trial, GroupX, StimulusA) - f(Trial, GroupY, StimulusB).$$

As an example of how group-stimulus contrast inferences work, suppose we want to find whether or not the PTSD group over-generalizes GS3 at trial 1. We would find the group-stimulus contrast by first obtaining the stimulus contrast of GS3 at trial 1 for the TC group and the stimulus contrast of GS3 at trial 1 for the PTSD group. We would then subtract the TC stimulus-contrast estimate from the PTSD stimulus-contrast estimate we obtained to look at the degree to which the PTSD group generalized the GS3 relative to the TC group at trial 1. As we define over-generalization as an increase in generalization in the PTSD (and SubPTSD) group compared to the healthy, TC group, we always subtracted the TC group's stimulus contrast estimates from the PTSD (and SubPTSD) group's stimulus contrast estimates. We did the same when comparing discrimination differences between groups. To compare the PTSD versus the SubPTSD group in generalization and discrimination, we subtracted the SubPTSD group's stimulus contrast estimates from the PTSD group's stimulus contrast estimates. Group-stimulus contrasts

were computed for each trial/stimulus contrast combination across the course of both the Acquisition and Generalization phases.

Our SSANOVA model used the Bayesian interpretation of a smoothing spline (Gu & Wahba, 1993; Wahba, 1983), so statistical inferences (i.e., confidence intervals and *p*-values) were based on the normal distribution. We tested differences in generalization between groups using 95% CIs; we also obtained *p*-values under the frequentist philosophy for those interested, which match results obtained by the 95% CIs. An outline of how to calculate 95% CIs for both the stimulus and group-stimulus contrasts can be found in Appendix C. Of note, we did not correct for multiple comparisons because the Bayesian confidence intervals have "across the function" coverage (Gu & Wahba, 1993; Wahba, 1983). Unstandardized effect sizes (i.e., differences between groups) as well as corresponding standard errors and 95% CIs are reported in tables to provide a fuller picture of the results.

Results

Pre-Acquisition Phase

In order to determine whether or not there were any stimulus or group effects before conditioning, we modeled risk appraisals, averaged across trials, during the Pre-acquisition phase using an SSANOVA with stimulus (6 levels: CS+, GS_3, GS_2, GS_1, oCS-, vCS-) and group (3 levels: PTSD, SubPTSD, TC) as predictors. The full SSANOVA model for the Pre-acquisition phase had an $R^2 = .01$, indicating that our model accounted for approximately 1% of the variance in risk appraisals during Pre-acquisition. An R^2 this small indicates that our predictor variables, stimulus and group,

had little to no predictive utility for estimating risk appraisals during the Pre-acquisition phase.

Acquisition Phase

Findings during the Acquisition phase of the study are of secondary interest and can be found in Appendix B below (see text, Tables AB1-AB3, and Figures AB1-AB2).

Generalization Phase

Our full SSANOVA model accounted for approximately 40% of the variance in risk appraisals (R^2 = .40) during the Generalization phase. A graph of fitted values (i.e., estimated risk appraisals) for each group, stimulus, and trial combination can be found in Figure AB3 of Appendix B. Using the SSANOVA model, estimated stimulus contrasts averaged across trials for each group revealed increased risk appraisals to CS+ versus oCS (PTSD: Estimated difference = 1.40, 95% CI [1.29, 1.52], SubPTSD: Estimated difference = 1.22, 95% CI [1.12, 1.32]; TC: Estimated difference = 1.00, 95% CI [0.91, 1.10]) and CS+ versus vCS- (PTSD: Estimated difference = 1.51, 95% CI [1.39, 1.62]; SubPTSD: Estimated difference = 1.34, 95% CI [1.23, 1.44]; TC: Estimated difference = 1.11, 95% CI [1.01, 1.21]), indicating that conditioning persisted during Generalization for each group. Two-tailed (mean difference) permutation tests demonstrated no significant differences in averaged risk appraisals between counterbalancing groups for CS+, oCS-, and vCS- throughout the Generalization phase (ps > .05).

Additionally, risk appraisals, as predicted by the stimulus-type main effect term only, fell along a relatively quadratic generalization gradient, with increasing stimulus similarity to CS+ corresponding to increases in risk appraisals (see Figure AB4 of

Appendix B). The 95% CIs for each stimulus type fell both above (CS+ and GS3) and below (vCS-, oCS-, GS1, and GS2) zero, reflecting a main effect of stimulus-type (i.e., evidence that the regression weight for the stimulus main effect term is not zero for all stimuli), with an increase in a stimulus' similarity to CS+ predicting an increase in risk appraisal. Estimated appraisals of risk, as predicted by the stimulus-type term only, fell along an expected generalization gradient, with a continual decrease in estimated risk appraisal from CS+ to GSs to oCS- to vCS-.

Group-stimulus contrasts.

Conditioned stimuli. Full trial by trial results (i.e., effect size estimates, associated 95% CIs, and statistical significance) for group differences in perceived risk to CS+ versus both oCS- and vCS- can be found in Figure 2 and Table 1 (see Table AB4 in Appendix B for PTSD versus SubPTSD). As can be seen, significantly elevated perceived risk to CS+, relative to vCS- and oCS-, was found in PTSD versus TC at trials 2-10. Additionally, significant elevations in SubPTSD versus TC were found at trials 3, 4, 9, and 10 for CS+ versus vCS-, and at trials 2, 3, 9 and 10 for CS+ versus oCS-.

Overall generalization. Full trial by trial results for group differences in overall generalization (all GSs averaged) can be found in Table 2 and Figure 3. While each of three groups showed declining levels of overall generalization across the 10 trials, reflective of discrimination learning (see Figure 3), larger group differences in generalization emerged at particular points in the learning record. Specifically, overall generalization was significantly elevated in PTSD versus TC at trials 1-7, but not at trials 8-10. Results thus indicate greater evidence of over-generalization of conditioned risk

appraisals in PTSD in early and middle trials but less evidence of over-generalization in PTSD toward the end of the learning record. The SubPTSD group showed a timecourse of group differences similar to that of PTSD, with significantly elevated overall generalization in SubPTSD versus TC at trials 2-5, but not at trial 1 or trials 6-10. Thus, like PTSD, over-generalization of conditioned risk appraisals in SubPTSD emerged in early and middle trials but resolved to levels that were non-significantly different from trauma controls later in the learning record. Despite similarities across PTSD and SubPTSD, over-generalization was slower to resolve in PTSD versus SubPTSD, with significant over-generalization extending through trial 7 in PTSD but only through trial 5 in SubPTSD. No significant differences in overall generalization were found between PTSD and SubPTSD at any trial.

Generalization to specific GSs. Full trial by trial results for group differences in generalization to GSs with high (GS_3), moderate (GS_2), and low (GS_1) resemblance to the conditioned threat-cue (CS+) can be found in Tables 3-4 and Figure 4. Results for PTSD versus SubPTSD can be found in Table AB5 in Appendix B.

GS_3. Those with PTSD, relative to TC, displayed significantly elevated generalization to GS_3 across all 10 trials, whereas significantly elevated generalization in those with SubPTSD, relative to TC, was limited to trials 1-6 and 9. Additionally, elevated generalization to GS_3 was observed in PTSD versus SubPTSD at trial 6 (see Table AB6). Such findings indicate that over-generalization to the GS most closely resembling CS+ was both more persistent across trials and somewhat more robust in PTSD versus SubPTSD.

GS_2. Those with PTSD, relative to TC, displayed significantly elevated generalization to GS_2 at trials 1-6, but not trials 7-10. Additionally, those with SubPTSD, relative to TC, displayed significantly elevated generalization to GS_2 at trials 2-4, but not trials 1 or 5-10. This pattern of results reflects greater evidence of over-generalization to the GS with moderate resemblance to CS+ among those with PTSD and SubPTSD toward the beginning of trials, with less evidence of over-generalization at later trials. Though group effects for PTSD and SubPTSD at GS_2 were characterized by a similar timecourse, elevated generalization to GS_2 was slower to resolve for PTSD versus SubPTSD, with significant over-generalization extending through trial 6 in PTSD but only through trial 4 in SubPTSD. Levels of generalization to GS_2 in PTSD versus SubPTSD did not differ at any trial (see Table AB6).

GS_1. No significant group differences in generalization to GS_1 emerged at any of the trials for PTSD versus TC, SubPTSD versus TC, or PTSD versus SubPTSD.

Discussion

The present study represents the first effort to model the trial by trial timecourse of PTSD-related over-generalization of threat expectancies to test the prediction that the expression of such over-generalization is not a stable marker of PTSD pathology but can be reduced with sufficient exposure to unreinforced generalization stimuli (GSs). Results largely support this hypothesis, with heightened overall generalization among those with PTSD versus trauma controls (TC) found in beginning and middle trials but not toward the end of the learning record. In other words, heightened levels of over-generalization are generally not *maintained* in PTSD throughout the generalization task. Those with

subthreshold PTSD (SubPTSD) showed a similar temporal course, with increases in overall generalization, relative to TC, found in the first but not second half of Generalization. Despite these similarities across PTSD and SubPTSD, heightened overall generalization persisted further into the learning record in PTSD versus SubPTSD suggesting that those with greater PTSD symptomatology require more learning trials to achieve levels of generalization more similar to those of healthy TCs.

Additionally, results for individual classes of GSs revealed unique timecourses for PTSD-related over-generalization to GSs with high (GS_3), moderate (GS_2), and low (GS_1) resemblance to the conditioned threat-cue (CS+). In PTSD, significant over-generalization persisted through all 10 trials for GS_3, resolved toward the end of the learning record for GS_2, and was not present at any of the 10 trials for GS_1. That overgeneralization to GS_3 in the PTSD group endured across all trials is contrary to predictions, and future work is needed to determine whether such over-generalization might resolve to levels more similar to TCs with additional GS_3 learning trials. In SubPTSD, significant over-generalization to GS_3 and GS_2 resolved by the middle and end of the learning record, respectively, and was not present at any trial for GS_1. This pattern of findings across PTSD and SubPTSD suggests that PTSD-related over-generalization is more persistent when evoked by stimuli with higher resemblance to a CS+ (GS_3), tends to resolve with sufficient trials when evoked by stimuli with moderate CS+ resemblance (GS_2), and is not present at any trial in response to stimuli with low resemblance to CS+ (GS_1). Though similar patterns of results were found for PTSD and SubPTSD, over-generalization to safe stimuli bearing high (GS_3) and moderate (GS_2) resemblance to CS+

among those with SubPTSD versus PTSD required fewer trials before reducing to levels that were non-significantly different from TCs.

Findings are generally consistent with the notion that expression of over-generalized conditioned fear is not a stable feature of PTSD but can be reduced with sufficient exposure to unreinforced generalization stimuli. While this is the first study to demonstrate reductions in PTSD-related over-generalization with repeated learning trials, several past fear-conditioning studies in anxiety and trauma-related disorders yield relatable findings. Specifically, elevated fear responding to safety cues resembling CS+ among those with PTSD and panic disorder have been shown to resolve during later stages of conditioning (Grillon & Morgan, 1999; Lissek et al., 2009; but see Jovanovic et al., 2010; Orr et al., 2000). Additionally, meta-analytic findings reflecting lab-based conditioning results across multiple case-control studies in PTSD found heightened fear reactivity to safety cues (CS-) resembling danger cues (CS+) among those with PTSD during the first half (acquisition training) but not the second half (extinction test) of unreinforced CS- trials (Duits et al., 2015). Finally, studies examining the timecourse of extinction have found elevated fear to the unreinforced CS+ only during early and middle extinction trials among those with PTSD (Fani et al., 2012; Norrholm et al., 2011; Norrholm et al., 2013) and those with more severe trauma-related re-experiencing symptoms (Norrholm et al., 2015). That is, PTSD symptoms were associated with slowed rates of safety learning to unreinforced CS+ presentations. Taken together, current and past findings suggest that those with PTSD are able to learn the safety value of both safe

GSs and CS+ no longer associated with aversive outcomes, but doing so requires increased exposure to unreinforced GSs and CS+, relative to healthy individuals.

Of important note, the term "stability" in the present discussion is used to describe the *maintenance* of over-generalization; this should be differentiated from a stable propensity of individuals with PTSD to initially over-generalize fear to stimuli resembling a threat cue prior to having sufficient exposure to unreinforced GSs to allow for a reduction in over-generalization. While the present study is concerned primarily with the maintenance of over-generalization, it also examines differences in initial propensity to over-generalize threat-related stimuli (i.e., trial 1 for GS stimuli). Our results demonstrate that PTSD and SubPTSD groups show an initial propensity to over-generalize GS_3 and GS_2 at trial 1 (although the effect isn't significant for the SubPTSD group at GS_2). The present study demonstrates the power of examining trial by trial analyses in conditioned fear generalization studies—it allows researchers to examine both 1) individual differences in the initial propensity to over-generalize and 2) individual differences in the maintenance of over-generalization. The ability to disentangle these two concepts has the potential to garner meaningful etiological insights, as initial propensity to over-generalize and maintenance of over-generalization may derive from different mechanisms.

Prediction Error as a Putative Mechanism for Prolonged Over-Generalization in PTSD

Prominent theories of classical conditioning (Pearce & Hall 1980; Rescorla & Wagner 1972) implicate *prediction error* as a key determinant of the associative strength

between a conditioned stimulus (CS) and an unconditioned stimulus (US). Through classical conditioning, the CS comes to signal the US, and subsequent presentations of the CS in the absence of the US create a prediction error, or a discrepancy between what was expected and what occurred. Such prediction errors promote learning by updating expectations of the US in the presence of the CS to increase the match between the expected and actual US outcome. In the current study, GSs elicit expectations of the US as a function of their perceptual similarity to CS+. This is particularly true for the first GS trial when participants have yet to experience GSs in the absence of the shock-US. The non-occurrence of shock during the first GS trial should thus elicit a prediction error, leading to a reduced expectancy of shock during the second GS trial. A similar effect should occur following each subsequent non-reinforced GS trial, leading to incremental decreases in perceived risk of shock across GS trials of the kind found in the current study (e.g., Figure 2). The heightened maintenance of shock expectancy across unreinforced GS trials found among those with PTSD and SubPTSD versus TC may therefore reflect a PTSD-related deficit in the efficient use of prediction errors to update GS-US associations.

Treatment Implications

The link between PTSD and slowed reductions in generalization of perceived risk prescribes a therapeutic approach aimed at reducing expectations of harm elicited by innocuous encounters that resemble aspects of the traumatic event, in addition to the actual features of the trauma. This could be achieved through in vivo and imaginal exposures to a *fear hierarchy* of trauma cues, with exposures to stimuli resembling

trauma cues added at each level of the hierarchy. Importantly, such exposures should aim to maximize prediction error by providing patients repeated contact with trauma cues and memories in the absence of feared outcomes while directing patients' attention to the resulting violation of expectations (e.g., Craske, Hermans, & Vervliet, 2018; Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014). Take, for example, an individual with combat-related PTSD who fears safe, roadside objects in their post-deployment environment that resemble roadside bombs encountered during combat. This patient may benefit from repeated exposures to different kinds of safe roadside objects, which vary in similarity to the encountered roadside bombs, in the absence of the predicted harm. The resulting prediction error should serve to disconfirm expectancies for threatening outcomes in the presence of safe roadside objects. In order to direct attention to exposure-elicited prediction errors, this patient would be asked to articulate what was predicted and what occurred, and the degree of "surprise" experienced following each exposure.

These kinds of exposure-based prediction errors should be repeatedly elicited until erroneous expectancies for aversive outcomes are reduced to a minimum, an approach that has thus far been shown to create more robust safety learning in panic disorder patients and individuals with high levels of panic symptoms than standard exposure techniques aimed at fear-reduction (e.g., Deacon et al., 2013; Salkovskis, Hackmann, Wells, Gelder, & Clark, 2007). Present results suggest that reductions in aversive expectancies for safe stimulus events resembling features of the trauma should be achievable in those with PTSD given a sufficient number of exposures and that

prolonged exposure regimens may be needed to reduce threat expectancies to innocuous stimuli with high resemblance to features of the trauma.

Limitations/Future Directions

There are a few limitations to the present study that we would like to present. First, we had a relatively small sample size. As outlined in Appendix C, smoothing splines in our model are selected via cross-validation (Craven & Wahba, 1978), which can help to reduce the overfitting that can be problematic with small sample sizes. That being said, replication of the present analysis should be done on samples with a larger *N*.

Second, our response variable, risk, is on an ordinal scale. For example, a rating of "1", indicating "moderate risk", could be seen by one participant as 30% chance of shock and another participant as 60% chance of shock. Therefore, risk appraisals, in reality, potentially have greater variance than what is recorded from our current scale. However, it should be noted that a vast majority of published literature demonstrating over-generalization in anxious groups use this three-point ordinal scale to procure cognitive appraisals of risk/threat vs. safety contingency of stimuli (e.g., Kaczkurkin et al., 2017, Lissek et al., 2014, Lissek et al., 2010). Future studies should aim to use a scale with more variation, such as what the participant believes is the "likelihood of shock" on a scale of 0% to 100%, to detect additional nuances in conditioning over time.

Lastly, the present study only examines *awareness* of stimulus contingency, not physiological measures of conditioning. The most common measures used to assess fear generalization are affective (physiological; fear- potentiated startle blinks as measured by electromyography (EMG)) and cognitive (appraisals of risk). Past research has shown

that cognitive awareness of stimulus contingencies and inhibition of fear-potentiated startle do not necessarily coincide (Jovanovic et al., 2006; Norrholm et al., 2006). Therefore, although we do see that fear over-generalization, as measured by cognitive awareness of stimulus contingencies, resolves later in the learning record, such a pattern may look different when examining PTSD/trauma control group differences in affective measures. To elucidate the process by which affective and cognitive measures of fear generalization relate to one another, future studies should investigate how these two measures change in temporal relation to one another across the course of the fear generalization task.

Conclusions

Current findings demonstrate that heightened generalization of threat expectancies in PTSD and subthreshold PTSD can be reduced to levels closer to those displayed by healthy trauma controls with sufficient learning trials. Additionally, more learning trials are required to reduce PTSD-related over-generalization of threat expectancies to safe stimuli bearing higher resemblance to the conditioned danger-cue. Such results support the use of prolonged courses of exposure therapy in PTSD that maximize violations of threat-related expectancies for safe stimulus events resembling the traumatic encounter.

Table 1

Trial by trial group-stimulus contrast statistics for conditioned stimuli (CS+ versus oCS- and vCS-) during Generalization for PTSD vs. trauma controls and subthreshold PTSD vs. trauma controls.

Contrast	Trial	PTSD vs. TC				SubPTSD vs. TC			
		Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs
CS+ vs. oCS-	1	0.259	0.158	1.63	[-0.05, 0.57]	0.241	0.153	1.58	[-0.06, 0.54]
	2	0.290	0.115	2.53*	[0.06, 0.52]	0.218	0.111	1.97*	[0.0004, 0.44]
	3	0.326	0.106	3.08*	[0.12, 0.53]	0.200	0.102	1.96*	[0.0001, 0.40]
	4	0.362	0.106	3.40*	[0.15, 0.57]	0.175	0.103	1.71	[-0.03, 0.38]
	5	0.390	0.108	3.61*	[0.18, 0.60]	0.146	0.104	1.41	[-0.06, 0.35]
	6	0.416	0.109	3.81*	[0.20, 0.63]	0.126	0.104	1.21	[-0.08, 0.33]
	7	0.438	0.109	4.00*	[0.22, 0.65]	0.135	0.103	1.31	[-0.07, 0.34]
	8	0.468	0.109	4.28*	[0.25, 0.68]	0.197	0.103	1.91	[-0.005, 0.40]
	9	0.512	0.120	4.28*	[0.28, 0.75]	0.294	0.112	2.63*	[0.07, 0.51]
	10	0.560	0.164	3.41*	[0.24, 0.88]	0.398	0.154	2.58*	[0.10, 0.70]
CS+ vs. vCS-	1	0.297	0.169	1.75	[-0.03, 0.63]	0.230	0.164	1.41	[-0.09, 0.55]
	2	0.335	0.122	2.74*	[0.10, 0.57]	0.228	0.118	1.94	[-0.002, 0.46]
	3	0.373	0.113	3.30*	[0.15, 0.59]	0.230	0.109	2.10*	[0.02, 0.44]
	4	0.404	0.114	3.55*	[0.18, 0.63]	0.216	0.110	1.96*	[0.0003, 0.43]
	5	0.414	0.116	3.59*	[0.19, 0.64]	0.184	0.111	1.67	[-0.03, 0.40]
	6	0.411	0.117	3.52*	[0.18, 0.64]	0.153	0.111	1.38	[-0.06, 0.37]
	7	0.403	0.117	3.44*	[0.17, 0.63]	0.148	0.111	1.34	[-0.07, 0.37]
	8	0.412	0.117	3.50*	[0.18, 0.64]	0.198	0.110	1.80	[-0.02, 0.41]
	9	0.444	0.128	3.48*	[0.19, 0.69]	0.286	0.119	2.41*	[0.05, 0.52]
	10	0.489	0.176	2.77*	[0.14, 0.83]	0.381	0.165	2.31*	[0.06, 0.70]

Estimates reflect unstandardized effects sizes for group differences in estimated risk appraisals across conditioned stimuli. *t*-values were obtained by taking the effect-size estimate divided by the standard error of the estimate. SubPTSD = subthreshold PTSD; TC = trauma control; CS+ = conditioned danger-cue; oCS- = circular conditioned safety-cue; vCS- = V-shaped conditioned safety-cue. *$p < .05$.

Table 2

Trial by trial group-stimulus contrast statistics reflecting group differences in overall generalization.

Trial	PTSD vs. TC				SubPTSD vs. TC				PTSD vs. SubPTSD			
	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs
1	0.299	0.134	2.23*	[0.04, 0.56]	0.235	0.130	1.81	[-0.02, 0.49]	0.064	0.135	0.47	[-0.20, 0.33]
2	0.324	0.097	3.33*	[0.13, 0.51]	0.238	0.094	2.53*	[0.05, 0.42]	0.086	0.098	0.88	[-0.11, 0.28]
3	0.340	0.089	3.82*	[0.17, 0.51]	0.237	0.086	2.75*	[0.07, 0.41]	0.103	0.090	1.15	[-0.07, 0.28]
4	0.334	0.090	3.71*	[0.16, 0.51]	0.219	0.087	2.52*	[0.05, 0.39]	0.115	0.090	1.27	[-0.06, 0.29]
5	0.297	0.092	3.25*	[0.12, 0.48]	0.180	0.088	2.05*	[0.01, 0.35]	0.118	0.092	1.28	[-0.06, 0.30]
6	0.244	0.092	2.64*	[0.06, 0.43]	0.135	0.088	1.53	[-0.04, 0.31]	0.109	0.093	1.18	[-0.07, 0.29]
7	0.191	0.092	2.07*	[0.01, 0.37]	0.107	0.087	1.22	[-0.06, 0.28]	0.085	0.092	0.92	[-0.10, 0.27]
8	0.161	0.092	1.75	[-0.02, 0.34]	0.111	0.087	1.27	[-0.06, 0.28]	0.051	0.092	0.55	[-0.13, 0.23]
9	0.162	0.102	1.59	[-0.04, 0.36]	0.137	0.095	1.45	[-0.05, 0.32]	0.024	0.102	0.24	[-0.18, 0.22]
10	0.181	0.140	1.29	[-0.09, 0.45]	0.170	0.131	1.3	[-0.09, 0.43]	0.011	0.140	0.08	[-0.26, 0.28]

Overall generalization is defined by estimated risk appraisals to all generalization stimuli (GS_1, GS_2, GS_3), averaged, minus risk appraisals to the V-shaped conditioned safety-cue (vCS-). Estimates reflect unstandardized effects sizes for group differences in overall generalization, with more positive estimates indicating greater overall generalization in PTSD versus trauma controls (TC), subthreshold PTSD (SubPTSD) versus TC, or PTSD versus SubPTSD. *t*-values were obtained by taking the estimate divided by the standard error of the estimate. *$p < .05$.

Table 3

Trial by trial group-stimulus contrast statistics reflecting PTSD versus trauma control differences in generalization to stimuli with high (GS_3), medium (GS_2), and low (GS_1) resemblance to the conditioned danger-cue.

Trial	GS_3				GS_2				GS_1			
	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs
1	0.387	0.158	2.44*	[0.08, 0.70]	0.338	0.153	2.20*	[0.04, 0.64]	0.174	0.145	1.20	[-0.11, 0.46]
2	0.439	0.115	3.83*	[0.21, 0.66]	0.362	0.112	3.24*	[0.14, 0.58]	0.172	0.106	1.61	[-0.04, 0.38]
3	0.482	0.105	4.57*	[0.27, 0.69]	0.374	0.102	3.69*	[0.18, 0.57]	0.163	0.096	1.71	[-0.02, 0.35]
4	0.498	0.106	4.68*	[0.29, 0.71]	0.362	0.102	3.53*	[0.16, 0.56]	0.141	0.096	1.47	[-0.05, 0.33]
5	0.476	0.108	4.40*	[0.26, 0.69]	0.316	0.105	3.02*	[0.11, 0.52]	0.100	0.099	1.02	[-0.09, 0.29]
6	0.43	0.109	3.93*	[0.22, 0.64]	0.252	0.106	2.39*	[0.05, 0.46]	0.050	0.099	0.51	[-0.14, 0.24]
7	0.381	0.109	3.48*	[0.17, 0.59]	0.187	0.105	1.79	[-0.02, 0.39]	0.005	0.098	0.05	[-0.19, 0.20]
8	0.352	0.110	3.22*	[0.14, 0.57]	0.149	0.105	1.42	[-0.06, 0.36]	-0.018	0.098	-0.18	[-0.21, 0.18]
9	0.354	0.120	2.96*	[0.12, 0.59]	0.145	0.116	1.24	[-0.08, 0.37]	-0.015	0.110	-0.13	[-0.23, 0.20]
10	0.374	0.165	2.27*	[0.05, 0.70]	0.162	0.159	1.02	[-0.15, 0.47]	0.005	0.149	0.04	[-0.29, 0.30]

Generalization is defined as estimated levels of risk to each generalization stimulus (GS) minus the V-shaped conditioned safety-cue (vCS-). Estimates reflect unstandardized effects sizes for PTSD versus trauma control (TC) differences in generalization to GS_3, GS_2, and GS_1, with more positive estimates indicating greater generalization in PTSD versus TC. *t*-values were obtained by taking the estimate divided by the standard error of the estimate. *$p < .05$.

Table 4

Trial by trial group-stimulus contrast statistics reflecting Subthreshold PTSD versus trauma control differences in generalization to stimuli with high (GS_3), medium (GS_2), and low (GS_1) resemblance to the conditioned danger-cue.

Trial	GS_3				GS_2				GS_1			
	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs
1	0.325	0.153	2.11*	[0.02, 0.63]	0.254	0.149	1.70	[-0.04, 0.55]	0.127	0.141	0.90	[-0.15, 0.40]
2	0.334	0.111	3.01*	[0.12, 0.55]	0.252	0.108	2.32*	[0.04, 0.46]	0.129	0.103	1.25	[-0.07, 0.33]
3	0.338	0.102	3.30*	[0.14, 0.54]	0.245	0.099	2.48*	[0.05, 0.44]	0.128	0.093	1.38	[-0.05, 0.31]
4	0.317	0.103	3.08*	[0.12, 0.52]	0.220	0.099	2.22*	[0.03, 0.41]	0.119	0.093	1.28	[-0.06, 0.30]
5	0.267	0.104	2.58*	[0.06, 0.47]	0.174	0.101	1.73	[-0.02, 0.37]	0.098	0.095	1.03	[-0.09, 0.28]
6	0.212	0.104	2.04*	[0.01, 0.42]	0.123	0.101	1.22	[-0.07, 0.32]	0.070	0.095	0.73	[-0.12, 0.26]
7	0.180	0.103	1.74	[-0.02, 0.38]	0.091	0.100	0.91	[-0.10, 0.29]	0.049	0.094	0.52	[-0.14, 0.23]
8	0.194	0.103	1.88	[-0.01, 0.40]	0.093	0.099	0.93	[-0.10, 0.29]	0.046	0.094	0.49	[-0.14, 0.23]
9	0.238	0.112	2.13*	[0.02, 0.46]	0.118	0.109	1.08	[-0.10, 0.33]	0.056	0.104	0.53	[-0.15, 0.26]
10	0.290	0.154	1.88	[-0.01, 0.59]	0.150	0.150	1.00	[-0.14, 0.44]	0.070	0.142	0.49	[-0.21, 0.35]

Generalization is defined as estimated levels of risk to each generalization stimulus (GS) minus the V-shaped conditioned safety-cue (vCS-). Estimates reflect unstandardized effects sizes for subthreshold PTSD (SubPTSD) versus trauma control (TC) differences in generalization to GS_3, GS_2, and GS_1, with more positive estimates indicating greater generalization in SubPTSD versus TC. *t*-values were obtained by taking the estimate divided by the standard error of the estimate. *$p < .05$.

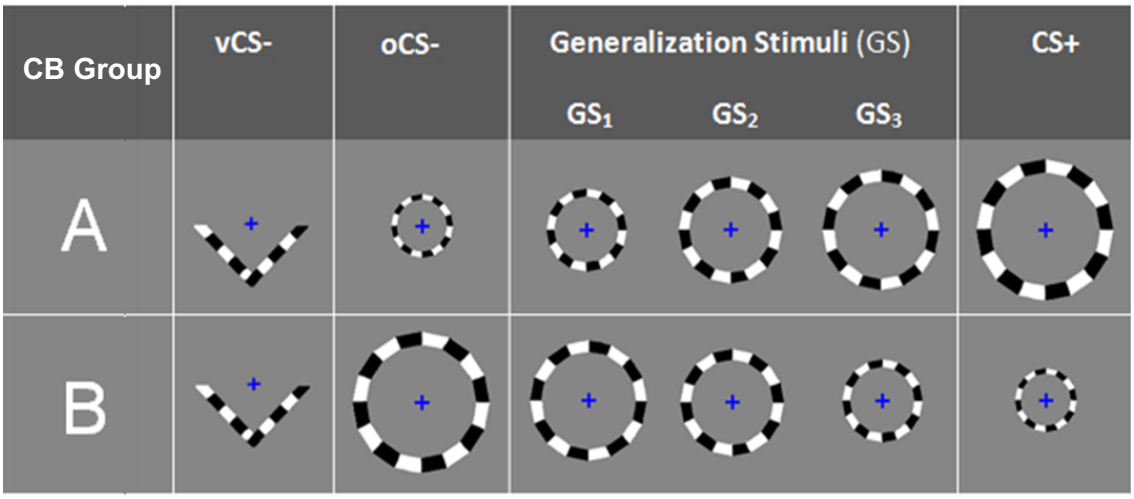

Figure 1. The stimuli used in the generalization task. CB = counterbalancing.

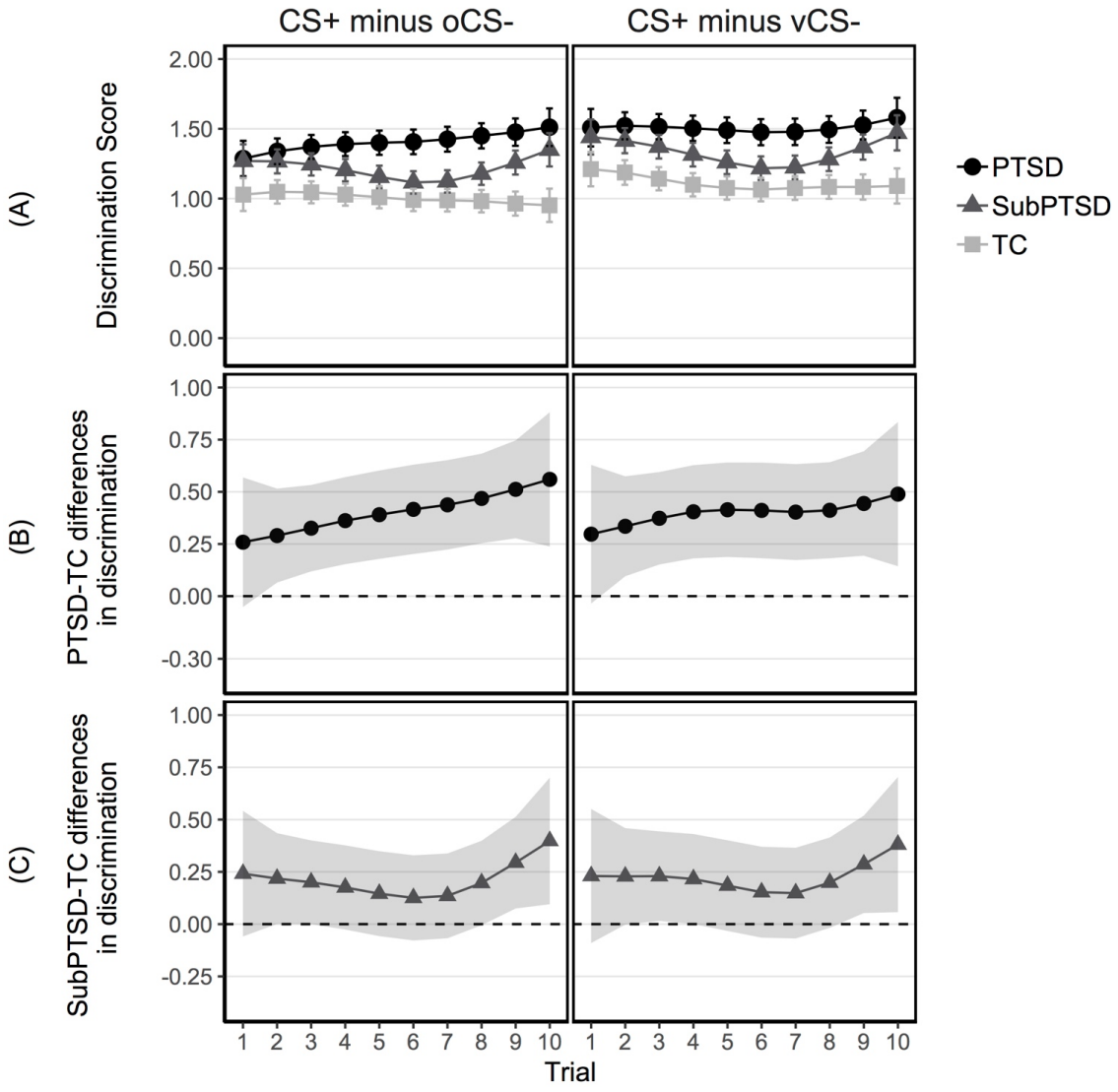

Figure 2. (A) Trial by trial levels of discriminative conditioning during the Generalization phase across groups. Discrimination is assessed by stimulus contrasts comparing risk appraisals to the conditioned danger-cue (CS+) versus both the circular and V-shaped conditioned safety-cues (oCS-, vCS-). Standard error bars accompany the estimates. (B) Results for PTSD versus trauma control (TC) group-stimulus contrasts reflecting group differences in discrimination across trials during Generalization. (C) Results for subthreshold PTSD (SubPTSD) versus TC group-stimulus contrasts reflecting group differences in discrimination across trials during Generalization. (B-C) Higher values indicate greater discrimination in PTSD or SubPTSD relative to TC. The shaded regions reflect 95% CIs, with lower-bound CIs that do not cross 0.00 indicating significant group effects.

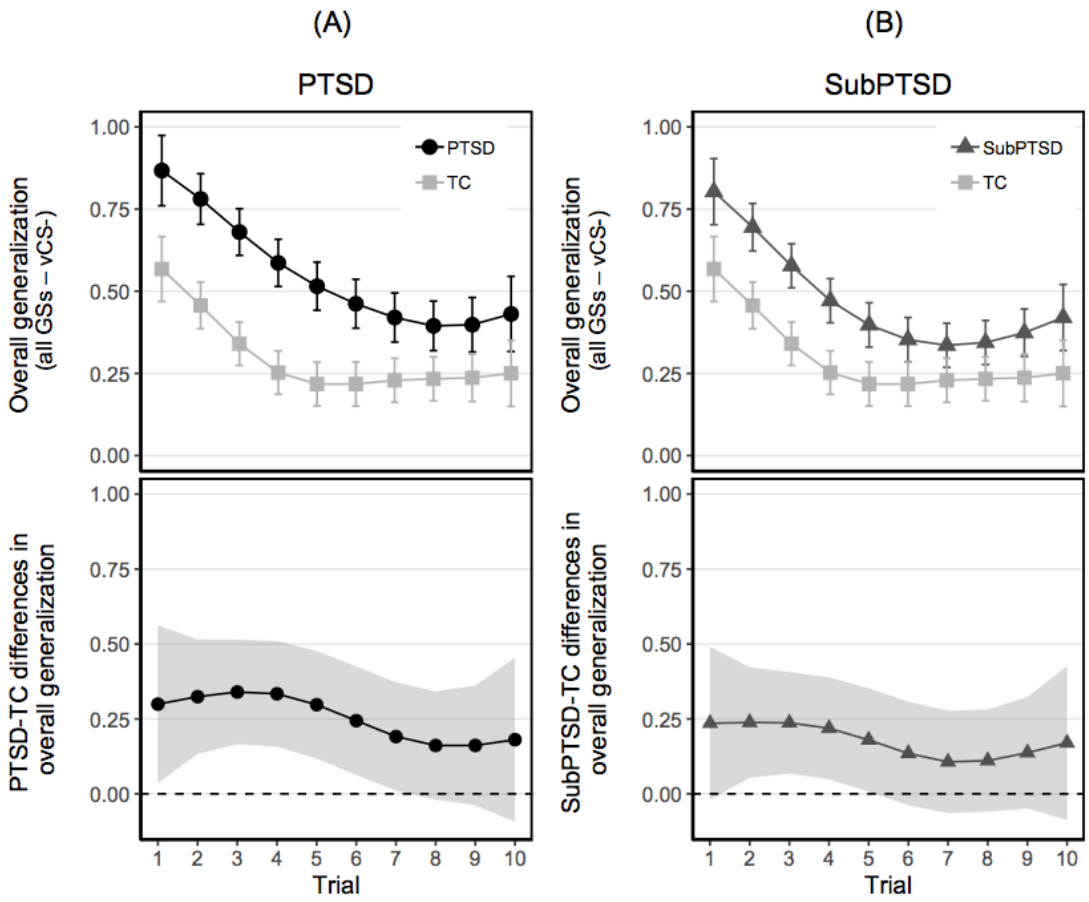

Figure 3. Trial by trial levels of overall generalization in (A) PTSD and (B) subthreshold PTSD (SubPTSD). Overall generalization is defined by stimulus contrasts assessing differences in estimated risk appraisals to all three generalization stimuli (GS_1, GS_2, GS_3) averaged versus estimated risk appraisals to the V-shaped conditioned safety-cue (vCS-). The top row of graphs plots levels of overall generalization and standard error bars across trials for each group, separately. The bottom row displays trial by trial results for group-stimulus contrasts reflecting (A) PTSD versus trauma controls (TC) and (B) SubPTSD versus TC differences in overall generalization, with higher values indicating greater overall generalization in PTSD or SubPTSD, relative to TC. The shaded regions reflect 95% CIs, with lower-bound CIs that do not cross 0.00 indicating significant group effects.

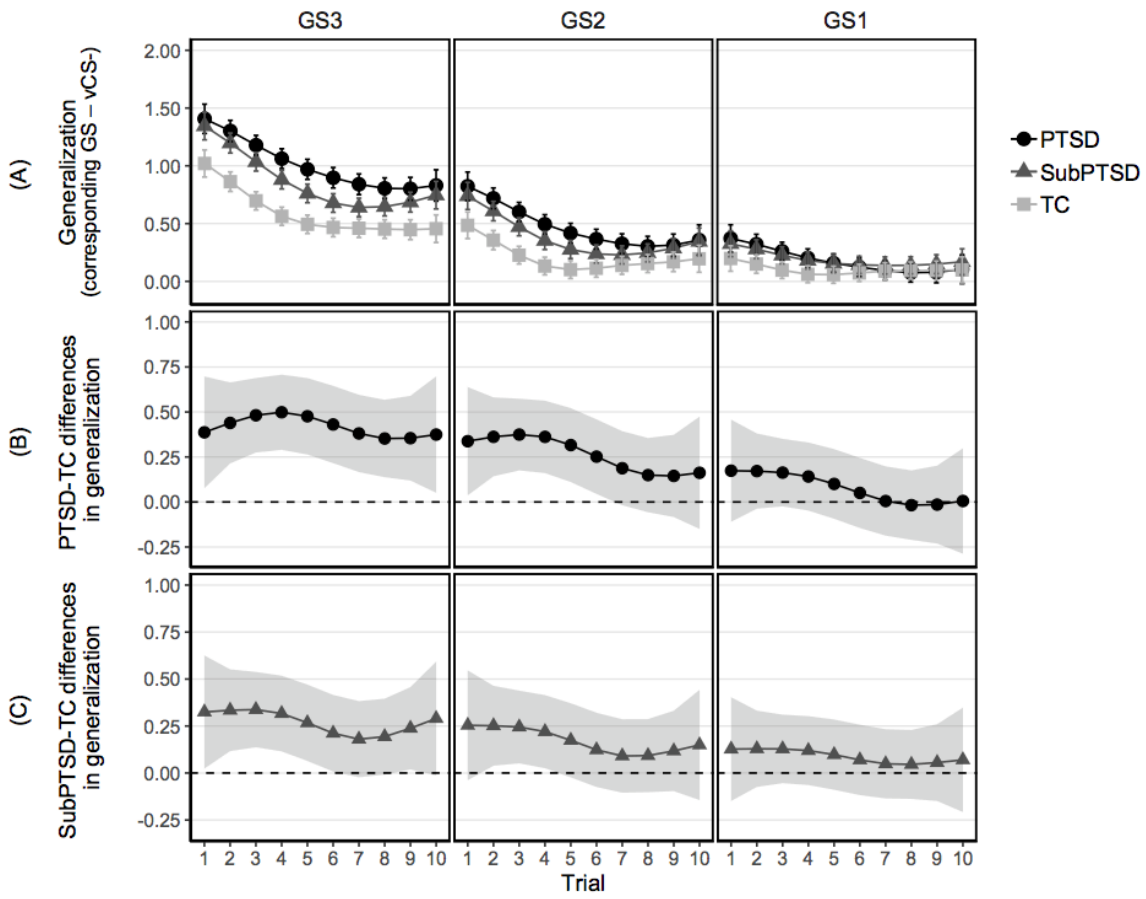

Figure 4. (A) Trial by trial levels of generalization to stimuli with high (GS_3), medium (GS_2), and low (GS_1) resemblance to the conditioned danger-cue across groups. Generalization is assessed by stimulus contrasts comparing estimated risk appraisals to each generalization stimulus versus the V-shaped conditioned safety-cue (vCS-). Standard error bars accompany the estimates. (B-C) Trial by trial group differences in generalization across (B) PTSD versus trauma controls (TC) and (C) Subthreshold PTSD (SubPTSD) versus TC for each of three generalization stimuli. Higher values indicate greater generalization in PTSD or SubPTSD, relative to TC. The shaded regions reflect 95% CIs, with lower-bound CIs that do not cross 0.00 indicating significant group effects.

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Appendix A - Participant Characteristics & Exclusion Criteria

Exclusion criteria included: a) history of Axis I psychiatric disorders before deployment, b) history of alcohol/substance abuse or dependence within the 6 months prior to study enrollment (barring nicotine), c) use of nicotine or caffeine on the day of testing, d) current use of mood stabilizers, anti-psychotics, anti-parkinsonian medication, anti-hypertensives, anticonvulsants, and alpha/beta adrenergic agents, e) current use of illegal substances, f) current Axis I psychiatric disorder for trauma controls, g) significant suicidal ideation/intent/behavior, h) having a medical implant, device, or condition that is not MRI safe, and i) medical conditions that interfered with study objectives. If participants were taking any medication on an "as needed" basis (e.g., stimulants, pain medications, benzodiazepines, sleep medications), they were excluded from the study unless they were able to forgo taking the medication 12 hours prior to testing without causing unnecessary symptom aggravation or impaired performance on study tasks and measures. Additionally, participants were excluded if they failed to condition as indicated by perceived risk of shock to the conditioned safety-cue that was equal to or higher than the conditioned danger-cue. Final participant characteristics can be found in Table AA1.

Table AA1

Participant characteristics.

Variable	PTSD (*n*=15)		Subthreshold (*n*=18)		Trauma Control (*n*=19)		Significance[1]
	Mean	SD	Mean	SD	Mean	SD	
Age	33.87	10.31	34.56	8.70	33.89	9.82	0.97[a]/0.98[b]
Education Level	4.93	0.88	5.11	1.57	5.32	1.60	0.74[a]/0.73[b]
STAI-State	47.27	11.40	42.50	11.08	32.89	9.55	<.001[a]/.001[b]
STAI-Trait	49.87	11.06	45.83	12.43	36.95	12.00	.007[a]/.008[b]
BDI	18.27	8.25	12.94	7.90	8.53	7.07	.003[a]/.002[b]
CAPS Total	60.93	16.19	30.94	7.97	13.84	6.56	<.001[a]/<.001[b]
	N	**%**	**N**	**%**	**N**	**%**	
Ethnicity							
African American	1	6.7%	1	5.56%	1	5.26%	
Caucasian	14	93.3%	17	94.44%	15	78.95%	
Hispanic	0	0.0%	0	0.0%	1	5.26%	
Asian Pacific	0	0.0%	0	0.0%	1	5.26%	
Unspecified	0	0.0%	0	0.0%	1	5.26%	

STAI – Spielberger State/Trait Anxiety Inventory (Spielberger, 1983); BDI-IA: Beck Depression Inventory (Beck & Steer, 1993); CAPS – Clinician Administered PTSD Scale for the DSM-IV (Blake et al., 1995); Education level was based on values as seen in the SCID-IV (Question NP110; First et al., 2001). [1]*p*-values denote the differences between groups and were obtained using a one-way ANOVA[a]/permutation test (max absolute mean difference)[b].

Appendix B – Additional Results

Acquisition Phase Results

Our full SSANOVA model accounted for approximately 39% of the variance in risk appraisals (R^2 = .39) during the Acquisition phase. A graph of the fitted values (i.e., estimated risk appraisals) generated by the SSANOVA model for each group, stimulus-type, and trial combination can be found in Figure A1. Additionally, the SSANOVA model indicated successful conditioning in each group, whether defining conditioning with contrasts comparing CS+ versus oCS- (PTSD: Estimated difference = 1.14, 95% CI [0.98, 1.30]; SubPTSD: Estimated difference = 1.07, 95% CI [0.93, 1.22]; TC: Estimated difference = 0.99, 95% CI [0.85, 1.14]) or contrasts comparing CS+ versus vCS- (PTSD: Estimated difference = 1.18, 95% CI [1.00, 1.36]; SubPTSD: Estimated difference = 1.18, 95% CI [1.01, 1.35]; TC: Estimated difference = 1.01, 95% CI [0.85, 1.18]). Two-tailed (mean difference) permutation tests demonstrated that there were no significant differences in averaged risk appraisals between counterbalance groups for CS+, oCS-, and vCS- throughout the Acquisition phase (*p*s > .05).

Results for estimated group-stimulus contrasts comparing PTSD to TC, SubPTSD to TC, and PTSD to SubPTSD can be found in Tables AB1, AB2, and AB3, respectively. Graphical results for the stimulus and group-stimulus contrasts can be found in Figure AB2. Group-stimulus contrasts compare group differences in discrimination of CS+ vs. vCS-, CS+ vs. oCS-, and oCS- vs. vCS- (i.e., stimulus contrasts) across the course of Acquisition. The "Estimate" column in each table reflects the estimated size of group differences in discrimination for each trial, with more positive estimates indicating

greater discrimination of CS+, from oCS- or vCS-, for the PTSD or SubPTSD group, relative to TCs. For PTSD versus SubPTSD contrasts, a more positive estimate indicates greater discrimination of CS+ from oCS- or vCS- in the PTSD group, relative to the SubPTSD group.

Discrimination between CS+ and oCS- was significantly elevated in PTSD versus TC at trials 3-5, but not at trials 1-2 and 6-8. Similarly, results show that discrimination between CS+ and vCS- was significantly elevated in PTSD versus TC at trials 3-5, but not at trials 1-2 and 6-8. Results thus indicate greater evidence of heightened discrimination of threat from safety cues in PTSD in middle trials but less evidence in early and late trials. There were no significant differences between the PTSD and TC groups in discrimination of oCS- vs. vCS- for all trials. There were also no significant differences between the SubPTSD and TC groups or PTSD and SubPTSD groups in discrimination of any pair of CSs for all trials.

Table AB1

Trial by trial group-stimulus contrast statistics for conditioned stimuli in PTSD versus trauma controls during Acquisition.

Contrast	Trial	Estimate	Std. Error	*t*	95% CIs
CS+ vs. oCS-	1	0.133	0.133	1.00	[-0.13, 0.39]
	2	0.186	0.099	1.88	[-0.01, 0.38]
	3	0.218	0.097	2.25*	[0.03, 0.41]
	4	0.226	0.102	2.22*	[0.03, 0.43]
	5	0.208	0.102	2.04*	[0.01, 0.41]
	6	0.171	0.097	1.76	[-0.02, 0.36]
	7	0.135	0.099	1.36	[-0.06, 0.33]
	8	0.103	0.133	0.78	[-0.16, 0.36]
CS+ vs. vCS-	1	0.119	0.166	0.72	[-0.21, 0.44]
	2	0.202	0.122	1.66	[-0.04, 0.44]
	3	0.261	0.120	2.17*	[0.03, 0.50]
	4	0.288	0.126	2.29*	[0.04, 0.53]
	5	0.271	0.126	2.16*	[0.02, 0.52]
	6	0.222	0.120	1.85	[-0.01, 0.46]
	7	0.166	0.121	1.37	[-0.07, 0.40]
	8	0.112	0.165	0.68	[-0.21, 0.44]
oCS- vs. vCS-	1	-0.013	0.133	-0.10	[-0.27, 0.25]
	2	0.017	0.099	0.17	[-0.18, 0.21]
	3	0.043	0.097	0.44	[-0.15, 0.23]
	4	0.061	0.102	0.60	[-0.14, 0.26]
	5	0.064	0.102	0.63	[-0.14, 0.26]
	6	0.051	0.097	0.53	[-0.14, 0.24]
	7	0.031	0.099	0.32	[-0.16, 0.22]
	8	0.009	0.133	0.07	[-0.25, 0.27]

Estimates reflect unstandardized effects sizes for group differences in estimated risk appraisals across conditioned stimuli. *t*-values were obtained by taking the effect-size estimate divided by the standard error of the estimate. CS+ = conditioned danger-cue; oCS- = circular conditioned safety-cue; vCS- = V-shaped conditioned safety-cue. *$p < .05$.

Table AB2

Trial by trial group-stimulus contrast statistics for conditioned stimuli in subthreshold PTSD versus trauma controls during Acquisition.

Contrast	Trial	Estimate	Std. Error	*t*	95% CIs
CS+ vs. oCS-	1	0.107	0.131	0.82	[-0.15, 0.36]
	2	0.118	0.097	1.22	[-0.07, 0.31]
	3	0.119	0.095	1.26	[-0.07, 0.31]
	4	0.110	0.100	1.11	[-0.08, 0.31]
	5	0.090	0.100	0.91	[-0.10, 0.29]
	6	0.069	0.095	0.72	[-0.12, 0.25]
	7	0.057	0.097	0.59	[-0.13, 0.25]
	8	0.056	0.131	0.43	[-0.20, 0.31]
CS+ vs. vCS-	1	0.182	0.164	1.11	[-0.14, 0.50]
	2	0.209	0.119	1.76	[-0.02, 0.44]
	3	0.219	0.117	1.87	[-0.01, 0.45]
	4	0.210	0.122	1.71	[-0.03, 0.45]
	5	0.180	0.122	1.47	[-0.06, 0.42]
	6	0.149	0.117	1.27	[-0.08, 0.38]
	7	0.133	0.118	1.13	[-0.10, 0.37]
	8	0.134	0.162	0.82	[-0.18, 0.45]
oCS- vs. vCS-	1	0.075	0.132	0.56	[-0.18, 0.33]
	2	0.091	0.097	0.94	[-0.10, 0.28]
	3	0.100	0.095	1.05	[-0.09, 0.29]
	4	0.099	0.100	0.99	[-0.10, 0.29]
	5	0.090	0.100	0.90	[-0.11, 0.28]
	6	0.080	0.095	0.84	[-0.11, 0.27]
	7	0.076	0.097	0.78	[-0.11, 0.27]
	8	0.077	0.131	0.59	[-0.18, 0.33]

Estimates reflect unstandardized effects sizes for group differences in estimated risk appraisals across conditioned stimuli. *t*-values were obtained by taking the effect-size estimate divided by the standard error of the estimate. CS+ = conditioned danger-cue; oCS- = circular conditioned safety-cue; vCS- = V-shaped conditioned safety-cue. *$p < .05$.

Table AB3

Trial by trial group-stimulus contrast statistics for conditioned stimuli in PTSD versus subthreshold PTSD during Acquisition.

Contrast	Trial	Estimate	Std. Error	*t*	95% CIs
CS+ vs. oCS-	1	0.026	0.134	0.19	[-0.24, 0.29]
	2	0.068	0.100	0.68	[-0.13, 0.26]
	3	0.099	0.098	1.02	[-0.09, 0.29]
	4	0.116	0.102	1.13	[-0.08, 0.32]
	5	0.117	0.102	1.15	[-0.08, 0.32]
	6	0.103	0.098	1.05	[-0.09, 0.29]
	7	0.077	0.100	0.78	[-0.12, 0.27]
	8	0.047	0.134	0.35	[-0.22, 0.31]
CS+ vs. vCS-	1	-0.062	0.168	-0.37	[-0.39, 0.27]
	2	-0.007	0.123	-0.06	[-0.25, 0.23]
	3	0.042	0.121	0.34	[-0.20, 0.28]
	4	0.078	0.127	0.62	[-0.17, 0.33]
	5	0.091	0.127	0.72	[-0.16, 0.34]
	6	0.074	0.121	0.61	[-0.16, 0.31]
	7	0.033	0.122	0.27	[-0.21, 0.27]
	8	-0.021	0.167	-0.13	[-0.35, 0.31]
oCS- vs. vCS-	1	-0.088	0.135	-0.65	[-0.35, 0.18]
	2	-0.075	0.100	-0.75	[-0.27, 0.12]
	3	-0.057	0.098	-0.59	[-0.25, 0.13]
	4	-0.038	0.102	-0.37	[-0.24, 0.16]
	5	-0.026	0.102	-0.25	[-0.23, 0.17]
	6	-0.029	0.097	-0.30	[-0.22, 0.16]
	7	-0.045	0.099	-0.45	[-0.24, 0.15]
	8	-0.068	0.134	-0.51	[-0.33, 0.19]

Estimates reflect unstandardized effects sizes for group differences in risk appraisals across conditioned stimuli. *t*-values were obtained by taking the effect-size estimate divided by the standard error of the estimate. CS+ = conditioned danger-cue; oCS- = circular conditioned safety-cue; vCS- = V-shaped conditioned safety-cue. *$p < .05$.

Table AB4

Trial by trial group-stimulus contrast statistics for conditioned stimuli (CS+ versus oCS- and vCS-) for PTSD versus subthreshold PTSD during Generalization.

Contrast	Trial	Estimate	Std. Error	*t*	95% CIs
CS+ vs. oCS-	1	0.017	0.160	0.11	[-0.30, 0.33]
	2	0.072	0.116	0.62	[-0.16, 0.30]
	3	0.126	0.106	1.18	[-0.08, 0.33]
	4	0.186	0.107	1.74	[-0.02, 0.40]
	5	0.245	0.109	2.25*	[0.03, 0.46]
	6	0.290	0.109	2.65*	[0.08, 0.50]
	7	0.302	0.109	2.77*	[0.09, 0.52]
	8	0.272	0.109	2.48*	[0.06, 0.49]
	9	0.218	0.120	1.82	[-0.02, 0.45]
	10	0.162	0.164	0.98	[-0.16, 0.48]
CS+ vs vCS-	1	0.067	0.171	0.39	[-0.27, 0.40]
	2	0.106	0.123	0.86	[-0.14, 0.35]
	3	0.144	0.114	1.26	[-0.08, 0.37]
	4	0.189	0.115	1.65	[-0.04, 0.41]
	5	0.230	0.116	1.98*	[0.002, 0.46]
	6	0.258	0.117	2.20*	[0.03, 0.49]
	7	0.255	0.117	2.17*	[0.02, 0.48]
	8	0.213	0.117	1.82	[-0.02, 0.44]
	9	0.158	0.128	1.24	[-0.09, 0.41]
	10	0.108	0.176	0.61	[-0.24, 0.45]

Estimates reflect unstandardized effects sizes for group differences in estimated risk appraisals across conditioned stimuli. *t*-values were obtained by taking the effect-size estimate divided by the standard error of the estimate. CS+ = conditioned danger-cue; oCS- = circular conditioned safety-cue; vCS- = V-shaped conditioned safety-cue. **p* < .05.

Table AB5

Trial by trial group-stimulus contrast statistics for each generalization stimulus for the PTSD versus subthreshold PTSD groups during Generalization.

Trial	GS_3				GS_2				GS_1			
	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs	Estimate	Std. Error	*t*	95% CIs
1	0.062	0.160	0.39	[-0.25, 0.38]	0.084	0.154	0.54	[-0.22, 0.39]	0.046	0.146	0.32	[-0.24, 0.33]
2	0.105	0.116	0.91	[-0.12, 0.33]	0.110	0.113	0.98	[-0.11, 0.33]	0.043	0.107	0.40	[-0.17, 0.25]
3	0.144	0.106	1.35	[-0.06, 0.35]	0.130	0.102	1.27	[-0.07, 0.33]	0.035	0.096	0.36	[-0.15, 0.22]
4	0.182	0.107	1.70	[-0.03, 0.39]	0.142	0.103	1.37	[-0.06, 0.34]	0.022	0.097	0.23	[-0.17, 0.21]
5	0.209	0.109	1.92	[-0.005, 0.42]	0.142	0.105	1.35	[-0.06, 0.35]	0.003	0.099	0.03	[-0.19, 0.20]
6	0.219	0.110	1.99*	[0.004, 0.43]	0.129	0.106	1.21	[-0.08, 0.34]	-0.020	0.100	-0.20	[-0.21, 0.18]
7	0.201	0.109	1.83	[-0.01, 0.42]	0.097	0.105	0.92	[-0.11, 0.30]	-0.044	0.098	-0.44	[-0.24, 0.15]
8	0.159	0.110	1.45	[-0.06, 0.37]	0.056	0.105	0.54	[-0.15, 0.26]	-0.063	0.098	-0.64	[-0.26, 0.13]
9	0.116	0.120	0.97	[-0.12, 0.35]	0.027	0.116	0.23	[-0.20, 0.26]	-0.070	0.110	-0.64	[-0.29, 0.15]
10	0.084	0.165	0.51	[-0.24, 0.41]	0.013	0.159	0.08	[-0.30, 0.32]	-0.065	0.149	-0.43	[-0.36, 0.23]

Estimates reflect unstandardized effects sizes for group differences in estimated risk appraisals for each generalization stimulus (GS), with more positive estimates indicating greater generalization in PTSD versus subthreshold PTSD (SubPTSD). *t*-values were obtained by taking the estimate divided by the standard error of the estimate. GS_3, GS_2, and GS_1 = generalization stimuli with high, medium, and low resemblance to the conditioned danger-cue. *$p < .05$.

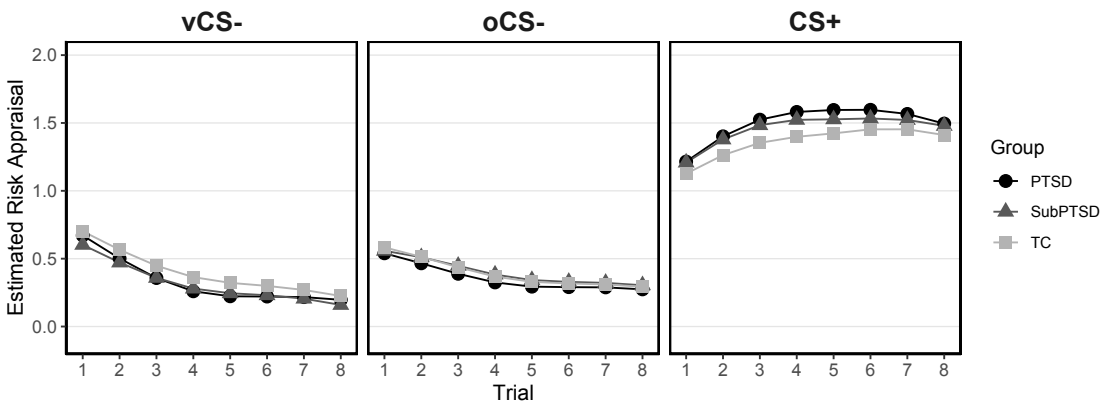

Figure AB1. Each graph displays the fitted values (i.e., estimated risk appraisals) of the SSANOVA model for the Acquisition phase for each group, stimulus, and trial combination. CS+ = conditioned danger-cue; oCS- = circular conditioned safety-cue; vCS- = V-shaped conditioned safety-cue; SubPTSD = subthreshold PTSD; TC = trauma control.

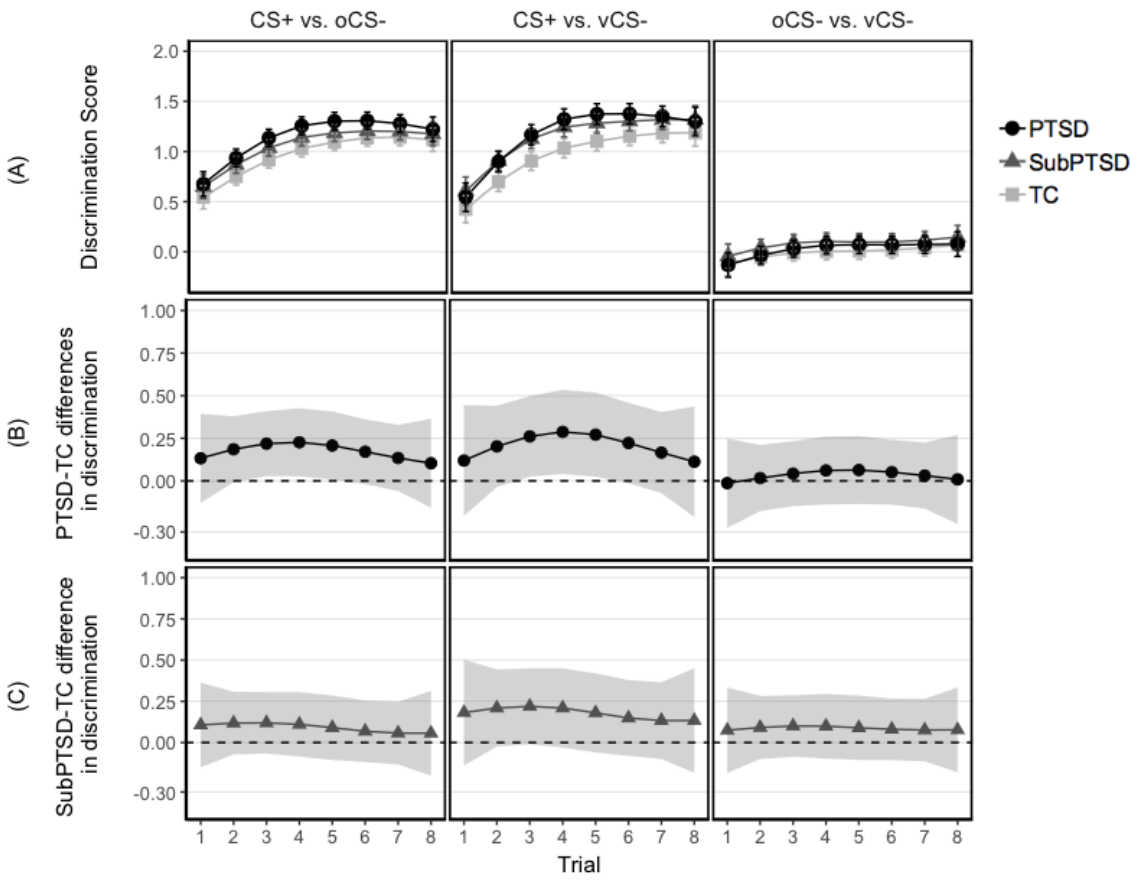

Figure AB2. (A) Trial by trial levels of discriminative conditioning during the Acquisition phase. Discrimination is assessed by stimulus contrasts comparing risk appraisals to the conditioned danger-cue (CS+) versus both the circular and V-shaped conditioned safety-cues (oCS-, vCS-). oCS- and vCS- contrasts are also included. Standard error bars accompany the estimates. (B) Results for PTSD versus trauma control (TC) group-stimulus contrasts reflecting group differences in discrimination across trials during Acquisition. (C) Results for subthreshold PTSD (SubPTSD) versus TC group-stimulus contrasts reflecting group differences in discrimination across trials during Acquisition. (B-C) Higher values indicate greater discrimination in PTSD or SubPTSD, relative to TC. The shaded regions reflect 95% CIs, with lower-bound CIs that do not cross 0.00 indicating significant group effects.

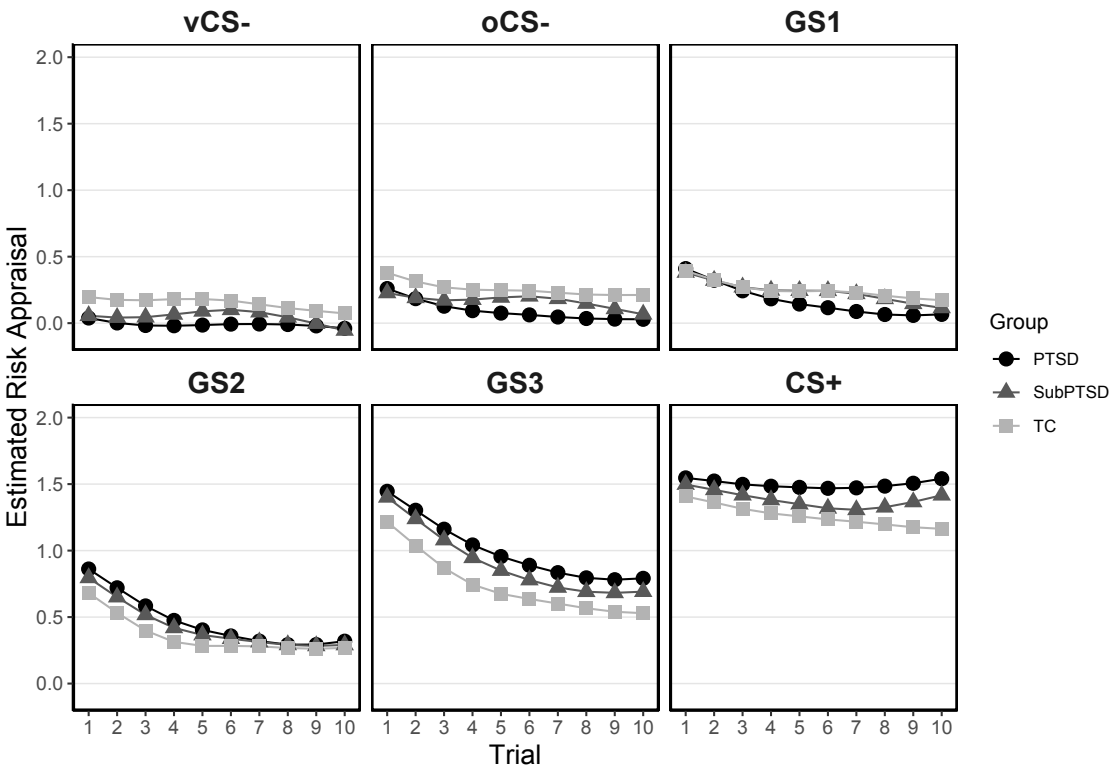

Figure AB3. Shows the fitted values (i.e., estimated risk appraisals) of the SSANOVA model for the Generalization phase for each group, stimulus, and trial combination. CS+ = conditioned danger-cue; vCS- = V-shaped conditioned safety-cue; oCS- = circular conditioned safety-cue; GS_3, GS_2, and GS_1 = generalization stimuli with high, medium, and low resemblance to the conditioned danger-cue; SubPTSD = subthreshold PTSD; TC = trauma control.

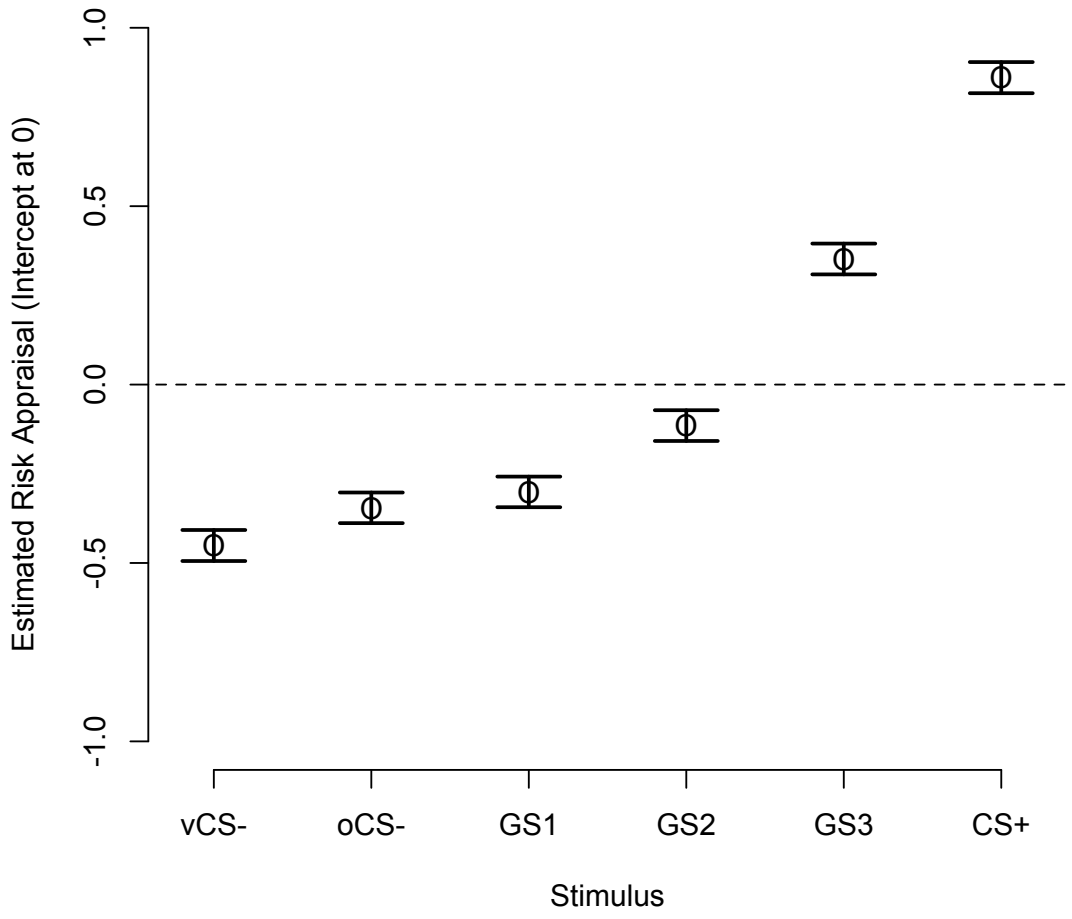

Figure AB4. Shows the estimated appraisal of risk using the stimulus main effect term of the Generalization phase model as the only predictor (i.e., intercept is excluded), with corresponding 95% confidence intervals around each estimate. Estimated appraisals of risk, as predicted by the stimulus main effect term only, fell along a relatively quadratic generalization gradient, with a continual decrease in risk appraisal from CS+ to GSs to oCS- to vCS-. The 95% CIs for each stimulus type fell both above (CS+ and GS_3) and below (vCS-, oCS-, GS_1, and GS_2) zero, reflecting a main effect of stimulus on the Generalization phase model.

Appendix C –Model Details

C.1 Overview of Smoothing Splines

Our mixed-effects nonparametric model was fit using a smoothing spline analysis of variance (SSANOVA; Gu, 2013). The SSANOVA model estimates unknown functions (smoothing splines), which relate our response variable to the predictor variables from the sample data. Unlike parametric regression, the SSANOVA model does not assume that the relationship between the response and predictor variables follows some predetermined (parametric) form; instead, the SSANOVA approach estimates the functional form of the relationship from the data itself (Gu, 2013). This aspect of nonparametric regression increases model flexibility, which is ideal for discovering the form of relationships among variables.

Using nonparametric regression, we can theoretically create a model that fits our sample data perfectly (i.e., has a mean-squared error of 0); however, this model with "perfect fit" will almost inevitability be more inaccurate when generalizing the model to future samples of data because it capitalizes on noise (James et al., 2013, pgs. 29-26). One critical aspect of the SSANOVA model is that it introduces a smoothing penalty to the model with the purpose of finding an ideal balance between fitting the sample data and the roughness of the estimated model function (Gu, 2013; Kimeldorf & Wahba, 1970). The variance and smoothing parameters for the present model were estimated using the two-stage approach described in Helwig (2016), which estimates the smoothing parameters via generalized cross-validation (Craven & Wahba, 1978) after estimating the variance parameters via restricted maximum likelihood estimation (Patterson &

Thompson, 1971). The main purpose of the cross-validation technique is to find a model that has the best chance at replication in future samples (James et al., 2013). In other words, it aims to protect against overfitting.

Figure AC1 illustrates the above points. Below are three graphs of the same data, with the black line indicating the underlying true function of how variable *y* and *x* are related in the population; the black dots represent a random sample of data drawn from the population. Each graph shows a different colored line that is a smoothing spline model fit using a specific smoothing parameter (SP). The first model uses no smoothing parameter, resulting in a model that fits the data perfectly. The second model uses a smoothing parameter of 1, resulting in a model that is almost linear. Lastly, the third model uses cross-validation (ordinary leave-one-out (LOO) method in this particular example case) to select the smoothing parameter, resulting in a model that is the closest to the underlying true function of the population.

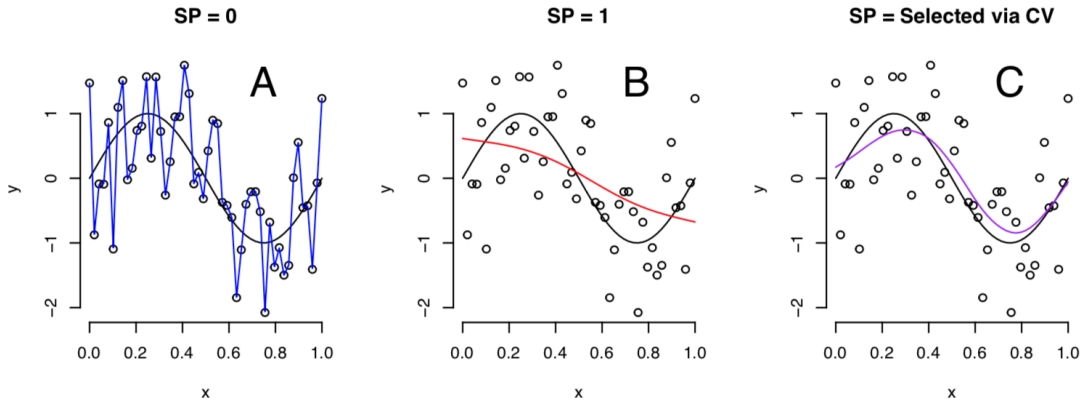

Figure AC1. Finding a model of the data with (A) no smoothing parameter (SP), (B) a smoothing parameter of 1, and (C) a smoothing parameter selected via ordinary leave-one-out cross-validation.

C.2 Functional Representation of the SSANOVA

Recall that our model takes on the form

$$y_{its} = f(t, g_i, s) + u_i + \epsilon_{its}$$

With y_{its} denoting the observed risk rating for the i-th subject at the t-th trial for the s-th stimulus, u_i denoting the random intercept for the i-th subject with group specific variance terms, and ϵ_{its} denoting the error term for the i-th subject at the t-th trial for the s-th stimulus. The SSANOVA represents the unknown function $f(\cdot)$ in terms of known basis functions, $\psi_j(\cdot)$, and unknown coefficients, β_j. The coefficients, β_j, define the linear combination of basis functions that produce $f(\cdot)$. If we let $z_i = (t, g_i, s)$, with (t, g_i, s) signifying a specific combination of the given levels of our predictor variables, $f(z_i)$ can be represented as

$$f(z_i) = \sum_{j=1}^{p} \psi_j(z_i)\beta_j.$$

Another manner in which this equation can be represented is

$$f(z_i) = \mathbf{x}_i'\boldsymbol{\beta}$$

where $\mathbf{x}_i' = [\psi_1(z_i), \ldots, \psi_p(z_i)]$ is a p x 1 vector of known basis functions to be evaluated at z_i, and $\boldsymbol{\beta}$ is a p x 1 vector containing basis function coefficients. The overall goal of the model is to estimate the unknown $\boldsymbol{\beta}$ coefficient vector.

C.3 Fitted Values of the SSANOVA Model

When the model is fit, we obtain our estimated $\boldsymbol{\beta}$ vector, which we will denote as $\widehat{\boldsymbol{\beta}}$**.** The covariance matrix of $\widehat{\boldsymbol{\beta}}$ is denoted by $\boldsymbol{\Sigma}_{\widehat{\boldsymbol{\beta}}}$. The fitted values of the model, denoted by $\hat{y}_i$, have the form

$$\hat{y}_i = \mathbf{x}_i'\widehat{\boldsymbol{\beta}}$$

and the variance of the fitted values can be obtained through

$$\mathrm{V}(\hat{y}_i) = \mathbf{x}_i'\boldsymbol{\Sigma}_{\widehat{\boldsymbol{\beta}}}\mathbf{x}_i$$

C.4 Stimulus Contrasts – Statistical Definition

The stimulus contrasts are modeled after the contrast methods used in Helwig, Shorter, Ma, & Hsiao-Wecksler (2016). A stimulus contrast is the difference between the

estimated risk ($\hat{y}_i$) between two different stimuli, s_1 and s_2, for the same trial and group. The stimulus contrast takes on the form

$$\hat{\delta}_{s_1,s_2} = (\mathbf{x}^{s_1} - \mathbf{x}^{s_2})' \widehat{\boldsymbol{\beta}}$$

where $\mathbf{x}^{s_1}$ is vector of basis functions to be evaluated at variable combination (t,g s_1) and $\mathbf{x}^{s_1}$ is vector of basis functions to be evaluated at variable combination (t,g s_2). The variance of the stimulus contrast estimate has a similar form to the fitted values

$$\mathrm{V}(\hat{\delta}_{s_1,s_2}) = (\mathbf{x}^{s_1} - \mathbf{x}^{s_2})' \boldsymbol{\Sigma}_{\widehat{\boldsymbol{\beta}}} (\mathbf{x}^{s_1} - \mathbf{x}^{s_2})$$

and the corresponding approximate 95% confidence interval has the form

$$\hat{\delta}_{s_1,s_2} \pm 1.96 \sqrt{\mathrm{V}(\hat{\delta}_{s_1,s_2})}$$

C.5 Group-Stimulus Contrasts – Statistical Definition

The group-stimulus contrasts compare the (model predicted) differences in stimulus contrasts between groups at a given trial. Before defining the group-stimulus contrast, we first want to specify a vector denoting the stimulus contrast for the first group as

$$\mathbf{d}_{g_1} = (\mathbf{x}_{\boldsymbol{g_1}}^{\boldsymbol{s_1}} - \mathbf{x}_{\boldsymbol{g_1}}^{\boldsymbol{s_2}})' \widehat{\boldsymbol{\beta}}$$

and for the second group of interest as

$$\mathbf{d}_{g_2} = (\mathbf{x}_{\boldsymbol{g_2}}^{\boldsymbol{s_1}} - \mathbf{x}_{\boldsymbol{g_2}}^{\boldsymbol{s_2}})' \widehat{\boldsymbol{\beta}}$$

with s_1 and s_2 indexing the two stimuli used for the stimulus contrast, g_1 indexing group 1, and g_2 indexing group 2. Given the two above equations, the estimated group-stimulus contrast takes on the form

$$\hat{\delta}_{g_1,g_2}^{s_1,s_2} = (\mathbf{d}_{g_1} - \mathbf{d}_{g_2})' \widehat{\boldsymbol{\beta}}$$

The variance of the contrast has the form

$$\mathrm{V}(\hat{\delta}_{g_1,g_2}^{s_1,s_2}) = (\mathbf{d}_{g_1} - \mathbf{d}_{g_2})' \boldsymbol{\Sigma}_{\widehat{\boldsymbol{\beta}}} (\mathbf{d}_{g_1} - \mathbf{d}_{g_2})$$

and the corresponding approximate 95% confidence interval has the form

$$\hat{\delta}_{g_1,g_2}^{s_1,s_2} \pm 1.96\sqrt{\mathrm{V}(\hat{\delta}_{g_1,g_2}^{s_1,s_2})}$$